amide-derivatized compound did not inhibit the overall plateau level of aggregation over time, the compound was not tested at concentrations higher than mole 33%. Higher concentrations of the amide-derivatized compound are predicted to inhibit the overall plateau level of aggregation, similar to cholyl-A.beta..sub.16-20 (PPI-350).

Detailed Description Paragraph Right (198):
The neurotoxicity of natural .beta.-amyloid peptide aggregates, in either the presence or absence of a .beta.-amyloid modulator, is tested in a cell-based assay using either a rat or human neuronally-derived cell line (PC-12 cells or NT-2 cells, respectively) and the viability indicator 3,(4,4-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT). (See e.g., Shearman, M. S. et al. (1994) Proc. Natl. Acad. Sci. USA 91:1470-1474; Hansen, M. B. et al. (1989) J. Immun. Methods 119:203-210 for a description of similar cell-based viability assays). PC-12 is a rat adrenal pheochromocytoma cell line and is available from the American Type Culture Collection, Rockville, Md. (ATCC CRL 1721). MTT (commercially available from Sigma Chemical Co.) is a chromogenic substrate that is converted from yellow to blue in viable cells, which can be detected spectrophotometrically.

Detailed Description Paragraph Right (201):
To determine the effect of a .beta.-amyloid modulator compound on the neurotoxicity of A.beta..sub.1-40 aggregates, a modulator compound, cholyl-A.beta..sub.6-20 (PPI-264), was preincubated with A.beta..sub.1-40 monomers under standard nucleation assay conditions as described in Example 6 and at particular time intervals post-incubation, aliquots of the .beta.-AP/modulator solution were removed and 1) the turbidity of the solution was assessed as a measure of aggregation and 2) the solution was applied to cultured neuronal cells for 48 hours at which time cell viability was assessed using MTT to determine the neurotoxicity of the solution. The results of the turbidity analysis are shown in FIG. 4, panels A, B and C. In panel A, A.beta..sub.1-40 and cholyl-A.beta..sub.6-20 were both present at 64 .mu.M. In panel B, A.beta..sub.1-40 was present at 30 .mu.M and cholyl-A.beta..sub.6-20 was present at 64 .mu.M. In panel C, A.beta..sub.1-40 was present at 10 .mu.M and cholyl-A.beta..sub.6-20 was present at 64 .mu.M. These data show that an equimolar amount of cholyl-A.beta..sub.6-20 is effective at inhibiting aggregation of A.beta..sub.1-40 (see FIG. 4, panel A) and that as the concentration of A.beta..sub.1-40 is reduced, the amount of detectable aggregation of the A.beta..sub.1-40 monomer is correspondingly reduced (compare FIG. 4, panels B and C with panel A). The corresponding results of the neurotoxicity analysis are shown in FIG. 4, panels D, E, and F. These results demonstrate that the .beta.-amyloid modulator compound not only inhibits aggregation of A.beta..sub.1-40 monomers but also inhibits the neurotoxicity of the A.beta..sub.1-40 solution, illustrated by the reduced percent toxicity of the cells when incubated with the A.beta..sub.1-40 /modulator solution as compared to A.beta..sub.1-40 alone (see e.g., FIG. 4, panel D). Moreover, even when A.beta..sub.1-40 aggregation was not detectable as measured by light scattering, the modulator compound inhibited the neurotoxicity of the A.beta..sub.1-40 solution (see FIG. 4, panels E and F). Thus, the formation of neurotoxic A.beta..sub.1-40 aggregates precedes the formation of insoluble aggregates detectable by light scattering and the modulator compound is effective at inhibiting the inhibiting the formation and/or activity of these neurotoxic aggregates. Similar results were seen with other modulator compounds, such as iminobiotinyl-A.beta..sub.6-20 (PPI-267), cholyl-A.beta..sub.16-20 (PPI-350) and cholyl-A.beta..sub.16-20 -amide (PPI-319).

Detailed Description Paragraph Left (11):
"n" can be, for example, an integer between 1 and 5, whereas "a" and "b" can be, for example, integers between 1 and 5. The modifying group "A" preferably comprises a cyclic, heterocyclic or polycyclic group. More preferably, A contains a cis-decalin group, such as cholanoyl structure or a cholyl group In other embodiments, A can comprise a biotin-containing group, a diethylene-triaminepentaacetyl group, a (-)-menthoxyacetyl group, a fluorescein-containing group or an N-acetylneuraminyl group. In yet other embodiments, the compound may promote aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides, may be further modified to alter a pharmacokinetic property of the compound or may be further modified to label the compound with a detectable substance.

Other Reference Publication (30):
LeVine, III, Harry (1993) "Thioflavine T Interaction with Synthetic Alzheimer's

Disease .beta.-Amyloid Peptides: Detection of Amyloid Aggregtion in Solution" Protein Science 2: 404-410.

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Brief Summary Paragraph Right (17):
The compounds of the invention can be further modified, for example to alter a pharmacokinetic property of the compound or to label the compound with a detectable substance. Preferred radioactive labels are radioactive iodine or technetium.

Brief Summary Paragraph Right (22):
In another embodiment, the invention provides a method for detecting the presence or absence of natural .beta.-amyloid peptides in a biological sample, comprising contacting a biological sample with a compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to thereby detect the presence or absence of natural .beta.-amyloid peptides in the biological sample. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine.

Brief Summary Paragraph Right (23):
In another embodiment, the invention provides a method for detecting natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease, comprising contacting a biological sample with a compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine. Preferably, the method facilitates diagnosis of Alzheimer's disease.

Detailed Description Paragraph Right (56):
Preferably, the compound inhibits aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides. In preferred embodiments, Xaa.sub.1 and Xaa.sub.2 are each phenylalanine structures or Xaa.sub.2 and Xaa.sub.3 are each phenylalanine structures. "n" can be, for example, an integer between 1 and 5, whereas "a" and "b" can be, for example, integers between 1 and 5. The modifying group "A" preferably comprises a cyclic, heterocyclic or polycyclic group. More preferably, A contains a cis-decalin group, such as cholanoyl structure or a cholyl group. In other embodiments, A can comprise a biotin-containing group, a diethylene-triaminepentaacetyl group, a (-)-menthoxyacetyl group, a fluorescein-containing group or an N-acetylneuraminyl group. In yet other embodiments, the compound may promotes aggregation of natural .beta.-amyloid peptides when contacted with the natural .beta.-amyloid peptides, may be further modified to alter a pharmacokinetic property of the compound or may be further modified to label the compound with a detectable substance.

Detailed Description Paragraph Right (60):
These specific compounds can be further modified to alter a pharmacokinetic property of the compound and/or further modified to label the compound with a detectable

substance.

Detailed Description Paragraph Right (61):
The modulator compounds of the invention can be incorporated into pharmaceutical compositions (described further in subsection V below) and can be used in detection and treatment methods as described further in subsection VI below.

Detailed Description Paragraph Right (72):
A .beta.-amyloid modulator compound of the invention can be further modified to alter the specific properties of the compound while retaining the ability of the compound to alter A.beta. aggregation and inhibit A.beta. neurotoxicity. For example, in one embodiment, the compound is further modified to alter a pharmacokinetic property of the compound, such as in vivo stability or half-life. In another embodiment, the compound is further modified to label the compound with a detectable substance. In yet another embodiment, the compound is further modified to couple the compound to an additional therapeutic moiety. Schematically, a modulator of the invention comprising an A.beta. aggregation core domain coupled directly or indirectly to at least one modifying group can be illustrated as MG-ACD, whereas this compound which has been further modified to alter the properties of the modulator can be illustrated as MG-ACD-CM, wherein CM represents an additional chemical modification.

Detailed Description Paragraph Right (74):
A modulator compound can be further modified to label the compound by reacting the compound with a detectable substance. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, .beta.-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include .sup.14 C, .sup.123 I, .sup.124 I, .sup.125 I, .sup.131 I, .sup.99m Tc, .sup.35 S or .sup.3 H. In a preferred embodiment, a modulator compound is radioactively labeled with .sup.14 C, either by incorporation of .sup.14 C into the modifying group or one or more amino acid structures in the modulator compound. Labeled modulator compounds can be used to assess the in vivo pharmacokinetics of the compounds, as well as to detect A.beta. aggregation, for example for diagnostic purposes. A.beta. aggregation can be detected using a labeled modulator compound either in vivo or in an in vitro sample derived from a subject.

Detailed Description Paragraph Right (81):
Another assay suitable for use in the screening method of the invention, a seeded extension assay, is also described further in Example 6. In this assay, .beta.-AP monomer and an aggregated .beta.-AP "seed" are combined, in the presence and absence of a test compound, and the amount of .beta.-fibril formation is assayed based on enhanced emission of the dye Thioflavine T when contacted with .beta.-AP fibrils. Moreover, .beta.-AP aggregation can be assessed by electron microscopy (EM) of the .beta.-AP preparation in the presence or absence of the modulator. For example, .beta. amyloid fibril formation, which is detectable by EM, is reduced in the presence of a modulator that inhibits .beta.-AP aggregation (i.e., there is a reduced amount or number of .beta.-fibrils in the presence of the modulator), whereas .beta. fibril formation is increased in the presence of a modulator that promotes .beta.-AP aggregation (i.e., there is an increased amount or number of .beta.-fibrils in the presence of the modulator).

Detailed Description Paragraph Right (97):
In the method of the invention, natural .beta. amyloid peptides can be contacted with a modulator either in vitro or in vivo. Thus, the term "contacted with" is intended to encompass both incubation of a modulator with a natural .beta.-AP preparation in vitro and delivery of the modulator to a site in vivo where natural .beta.-AP is present. Since the modulator compound interacts with natural .beta.-AP, the modulator compounds can be used to detect natural .beta.-AP, either in vitro or in vivo. Accordingly, one use of the modulator compounds of the invention is as diagnostic agents to detect the presence of natural .beta.-AP, either in a biological sample or in vivo in a subject. Furthermore, detection of natural .beta.-AP utilizing a modulator compound of the

invention further can be used to diagnose amyloidosis in a subject. Additionally, since the modulator compounds of the invention disrupt .beta.-AP aggregation and inhibit .beta.-AP neurotoxicity, the modulator compounds also are useful in the treatment of disorders associated with .beta.-amyloidosis, either prophylactically or therapeutically. Accordingly, another use of the modulator compounds of the invention is as therapeutic agents to alter aggregation and/or neurotoxicity of natural .beta.-AP.

Detailed Description Paragraph Right (98):
In one embodiment, a modulator compound of the invention is used in vitro, for example to detect and quantitate natural .beta.-AP in sample (e.g., a sample of biological fluid). To aid in detection, the modulator compound can be modified with a detectable substance. The source of natural .beta.-AP used in the method can be, for example, a sample of cerebrospinal fluid (e.g., from an AD patient, an adult susceptible to AD due to family history, or a normal adult). The natural .beta.-AP sample is contacted with a modulator of the invention and aggregation of the .beta.-AP is measured, such as by as assay described in Examples 2, 5 and 6. Preferably, the nucleation assay and/or seeded extension assay described in Example 6 is used. The degree of aggregation of the .beta.-AP sample can then be compared to that of a control sample(s) of a known concentration of .beta.-AP, similarly contacted with the modulator and the results can be used as an indication of whether a subject is susceptible to or has a disorder associated with .beta.-amyloidosis. Moreover, .beta.-AP can be detected by detecting a modulating group incorporated into the modulator. For example, modulators incorporating a biotin compound as described herein (e.g., an amino-terminally biotinylated .beta.-AP peptide) can be detected using a streptavidin or avidin probe which is labeled with a detectable substance (e.g., an enzyme, such as peroxidase). Detection of natural .beta.-AP aggregates mixed with a modulator of the invention using a probe that binds to the modulating group (e.g., biotin/streptavidin) is described further in Example 2.

Detailed Description Paragraph Right (99):
In another embodiment, a modulator compound of the invention is used in vivo to detect, and, if desired, quantitate, natural .beta.-AP deposition in a subject, for example to aid in the diagnosis of .beta. amyloidosis in the subject. To aid in detection, the modulator compound can be modified with a detectable substance, preferably .sup.99m Tc or radioactive iodine (described further above), which can be detected in vivo in a subject. The labeled .beta.-amyloid modulator compound is administered to the subject and, after sufficient time to allow accumulation of the modulator at sites of amyloid deposition, the labeled modulator compound is detected by standard imaging techniques. The radioactive signal generated by the labeled compound can be directly detected (e.g., whole body counting), or alternatively, the radioactive signal can be converted into an image on an autoradiograph or on a computer screen to allow for imaging of amyloid deposits in the subject. Methods for imaging amyloidosis using radiolabeled proteins are known in the art. For example, serum amyloid .beta. component (SAP), radiolabeled with either .sup.123 I or .sup.99m Tc, has been used to image systemic amyloidosis (see e.g., Hawkins, P. N. and Pepys, M. B. (1995) Eur. J Nucl. Med. 22:595-599). Of the various isotypes of radioactive iodine, preferably .sup.123 I (half-life=13.2 hours) is used for whole body scintigraphy, .sup.124 I (half life=4 days) is used for positron emission tomography (PET), .sup.125 I (half life=60 days) is used for metabolic turnover studies and .sup.131 I (half life=8 days) is used for whole body counting and delayed low resolution imaging studies. Analogous to studies using radiolabeled SAP, a labeled modulator compound of the invention can be delivered to a subject by an appropriate route (e.g., intravenously, intraspinally, intracerebrally) in a single bolus, for example containing 100 .mu.g of labeled compound carrying approximately 180 MBq of radioactivity.

Detailed Description Paragraph Right (100):
The invention provides a method for detecting the presence or absence of natural .beta.-amyloid peptides in a biological sample, comprising contacting a biological sample with a compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to thereby detect the presence or absence of natural .beta.-amyloid peptides in the biological sample. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the

biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine.

Detailed Description Paragraph Right (101):
The invention also provides a method for detecting natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease, comprising contacting a biological sample with the compound of the invention and detecting the compound bound to natural .beta.-amyloid peptides to facilitate diagnosis of a .beta.-amyloidogenic disease. In one embodiment, the .beta.-amyloid modulator compound and the biological sample are contacted in vitro. In another embodiment, the .beta.-amyloid modulator compound is contacted with the biological sample by administering the .beta.-amyloid modulator compound to a subject. For in vivo administration, preferably the compound is labeled with radioactive technetium or radioactive iodine. Preferably, use of the method facilitates diagnosis of Alzheimer's disease.

Detailed Description Paragraph Right (132):
Different amyloids are characterized by the type of protein(s) or peptide(s) present in the deposit. For example, as described hereinbefore, amyloid deposits associated with Alzheimer's disease comprise the .beta.-amyloid peptide and thus a modulator compound of the invention for detecting and/or treating Alzheimer's disease is designed based on modification of the .beta.-amyloid peptide. The identities of the protein(s) or peptide(s) present in amyloid deposits associated with a number of other amyloidogenic diseases have been elucidated. Accordingly, modulator compounds for use in the detection and/or treatment of these other amyloidogenic diseases can be prepared in a similar fashion to that described herein for .beta.-AP-derived modulators. In vitro assay systems can be established using an amyloidogenic protein or peptide which forms fibrils in vitro, analogous to the A.beta. assays described herein. Modulators can be identified using such assay systems, based on the ability of the modulator to disrupt the .beta.-sheet structure of the fibrils. Initially, an entire amyloidogenic protein can be modified or, more preferably, a peptide fragment thereof that is known to form fibrils in vitro can be modified (e.g., analogous to A.beta.1-40 described herein). Amino acid deletion and substitution analyses can then be performed on the modified protein or peptide (analogous to the studies described in the Examples) to delineate an aggregation core domain that is sufficient, when modified, to disrupt fibril formation.

Detailed Description Paragraph Right (134):
Transthyretin (TTR)--Amyloids containing transthyretin occur in familial amyloid polyneuropathy (Portuguese, Japanese and Swedish types), familial amyloid cardiomyopathy (Danish type), isolated cardiac amyloid and systemic senile amyloidosis. Peptide fragments of transthyretin have been shown to form amyloid fibrils in vitro. For example, TTR 10-20 and TTR 105-115 form amyloid-like fibrils in 20-30% acetonitrile/water at room temperature (Jarvis, J. A., et al. (1994) Int. J. Pept. Protein Res. 44:388-398). Moreover, familial cardiomyopathy (Danish type) is associated with mutation of Leu at position 111 to Met, and an analogue of TTR 105-115 in which the wildtype Leu at position 111 has been substituted with Met (TTR 105-115Met111) also forms amyloid-like fibrils in vitro (see e.g., Hermansen, L. F., et al. (1995) Eur. J. Biochem. 227:772-779; Jarvis et al. supra). Peptide fragments of TTR that form amyloid fibrils in vitro are also described in Jarvis, J. A., et al. (1993) Biochem. Biophys. Res. Commun. 192:991-998 and Gustavsson, A., et al. (1991) Biochem. Biophys. Res. Commun. 175:1159-1164. A peptide fragment of wildtype or mutated transthyretin that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of familial amyloid polyneuropathy (Portuguese, Japanese and Swedish types), familial amyloid cardiomyopathy (Danish type), isolated cardiac amyloid or systemic senile amyloidosis.

Detailed Description Paragraph Right (135):
Prion Protein (PrP)--Amyloids in a number of spongiform encephalopathies, including scrapie in sheep, bovine spongiform encephalopathy in cows and Creutzfeldt-Jakob disease (CJ) and Gerstmann-Straussler-Scheinker syndrome (GSS) in humans, contain PrP. Limited proteolysis of PrPSc (the prion protein associated with scrapie) leads to a 27-30 kDa fragment (PrP27-30) that polymerizes into rod-shaped amyloids (see e.g., Pan, K. M., et al. (1993) Proc. Natl. Acad. Sci. USA 90:10962-10966; Gasset, M., et

al. (1993) Proc. Natl. Acad. Sci. USA 90:1-5). Peptide fragments of PrP from humans and other mammals have been shown to form amyloid fibrils in vitro. For example, polypeptides corresponding to sequences encoded by normal and mutant alleles of the PRNP gene (encoding the precursor of the prion protein involved in CJ), in the regions of codon 178 and codon 200, spontaneously form amyloid fibrils in vitro (see e.g., Goldfarb, L. G., et al. (1993) Proc. Natl. Acad. Sci. USA 90:4451-4454). A peptide encompassing residues 106-126 of human PrP has been reported to form straight fibrils similar to those extracted from GSS brains, whereas a peptide encompassing residues 127-147 of human PrP has been reported to form twisted fibrils resembling scrapie-associated fibrils (Tagliavini, F., et al. (1993) Proc. Natl. Acad. Sci. USA 90:9678-9682). Peptides of Syrian hamster PrP encompassing residues 109-122, 113-127, 113-120, 178-191 or 202-218 have been reported to form amyloid fibrils, with the most amyloidogenic peptide being Ala-Gly-Ala-Ala-Ala-Ala-Gly-Ala (SEQ ID NO:17), which corresponds to residues 113-120 of Syrian hamster PrP but which is also conserved in PrP from other species (Gasset, M., et al. (1992) Proc. Natl. Acad. Sci. USA 89:10940-10944). A peptide fragment of PrP that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of scrapie, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease or Gerstmann-Straussler-Scheinker syndrome.

Detailed Description Paragraph Right (136):
Islet Amyloid Polypeptide (IAPP, also known as amylin)--Amyloids containing IAPP occur in adult onset diabetes and insulinoma. IAPP is a 37 amino acid polypeptide formed from an 89 amino acid precursor protein (see e.g., Betsholtz, C., et al. (1989) Exp. Cell. Res. 183:484-493; Westermark, P., et al. (1987) Proc. Natl. Acad. Sci. USA 84:3881-3885). A peptide corresponding to IAPP residues 20-29 has been reported to form amyloid-like fibrils in vitro, with residues 25-29, having the sequence Ala-Ile-Leu-Ser-Ser (SEQ ID NO:18), being strongly amyloidogenic (Westermark, P., et al. (1990) Proc. Natl. Acad. Sci. USA 87:5036-5040; Glenner, G. G., et al. (1988) Biochem. Biophys. Res. Commun. 155:608-614). A peptide fragment of IAPP that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of adult onset diabetes or insulinoma.

Detailed Description Paragraph Right (137):
Atrial Natriuretic Factor (ANF)--Amyloids containing ANF are associated with isolated atrial amyloid (see e.g., Johansson, B., et al. (1987) Biochem. Biophys. Res. Commun. 148:1087-1092). ANF corresponds to amino acid residues 99-126 (proANF99-126) of the ANF prohormone (proANP1-126) (Pucci, A., et al. (1991) J. Pathol. 165:235-241). ANF, or a fragment thereof, that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of isolated atrial amyloid.

Detailed Description Paragraph Right (138):
Kappa or Lambda Light Chain--Amyloids containing kappa or lambda light chains are associated idiopathic (primary) amyloidosis, myeloma or macroglobulinemia-associated amyloidosis, and primary localized cutaneous nodular amyloidosis associated with Sjogren's syndrome. The structure of amyloidogenic kappa and lambda light chains, including amino acid sequence analysis, has been characterized (see e.g., Buxbaum, J. N., et al. (1990) Ann. Intern. Med. 112:455-464; Schormann, N., et al. (1995) Proc. Natl. Acad. Sci. USA 92:9490-9494; Hurle, M. R., et al. (1994) Proc. Natl. Acad. Sci. USA 91:5446-5450; Liepnieks, J. J., et al. (1990) Mol. Immunol. 27:481-485; Gertz, M. A., et al. (1985) Scand. J. Immunol. 22:245-250; Inazumi, T., et al. (1994) Dermatology 189:125-128). Kappa or lambda light chains, or a peptide fragment thereof that forms amyloid fibrils, can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of idiopathic (primary) amyloidosis, myeloma or macroglobulinemia-associated amyloidosis or primary localized cutaneous nodular amyloidosis associated with Sjogren's syndrome.

Detailed Description Paragraph Right (139):
Amyloid A--Amyloids containing the amyloid A protein (AA protein), derived from serum amyloid A, are associated with reactive (secondary) amyloidosis (see e.g., Liepnieks, J. J., et al. (1995) Biochim. Biophys. Acta 1270:81-86), familial Mediterranean Fever and familial amyloid nephropathy with urticaria and deafness (Muckle-Wells syndrome) (see e.g., Linke, R. P., et al. (1983) Lab. Invest. 48:698-704). Recombinant human

serum amyloid A forms amyloid-like fibrils in vitro (Yamada, T., et al. (1994) Biochim. Biophys. Acta 1226:323-329) and circular dichroism studies revealed a predominant .beta. sheet/turn structure (McCubbin, W. D., et al. (1988) Biochem J. 256:775-783). Serum amyloid A, amyloid A protein or a fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of reactive (secondary) amyloidosis, familial Mediterranean Fever and familial amyloid nephropathy with urticaria and deafness (Muckle-Wells syndrome).

Detailed Description Paragraph Right (140):
Cystatin C--Amyloids containing a variant of cystatin C are associated with hereditary cerebral hemorrhage with amyloidosis of Icelandic type. The disease is associated with a leucine to glycine mutation at position 68 and cystatin C containing this mutation aggregates in vitro (Abrahamson, M. and Grubb, A. (1994) Proc. Natl. Acad. Sci. USA 91:1416-1420). Cystatin C or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of hereditary cerebral hemorrhage with amyloidosis of Icelandic type.

Detailed Description Paragraph Right (141):
.beta.2 microglobulin--Amyloids containing .beta.2 microglobulin (.beta.2M) are a major complication of long term hemodialysis (see e.g., Stein, G., et al. (1994) Nephrol. Dial. Transplant. 9:48-50; Floege, J., et al. (1992) Kidney Int. Suppl. 38:S78-S85; Maury, C. P. (1990) Rheumatol. Int. 10:1-8). The native .beta.2M protein has been shown to form amyloid fibrils in vitro (Connors, L. H., et al. (1985) Biochem. Biophys. Res. Commun. 131:1063-1068; Ono, K., et al. (1994) Nephron 66:404-407). .beta.2M, or a peptide fragment thereof that forms amyloid fibrils, can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of amyloidosis associated with long term hemodialysis.

Detailed Description Paragraph Right (142):
Apolipoprotein A-I (ApoA-I)--Amyloids containing variant forms of ApoA-I have been found in hereditary non-neuropathic systemic amyloidosis (familial amyloid polyneuropathy III). For example, N-terminal fragments (residues 1-86, 1-92 and 1-93) of an ApoA-I variant having a Trp to Arg mutation at position 50 have been detected in amyloids (Booth, D. R., et al. (1995) QJM 88:695-702). In another family, a leucine to arginine mutation at position 60 was found (Soutar, A. K., et al. (1992) Proc. Natl. Acad. Sci. USA 89:7389-7393). ApoA-I or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of hereditary non-neuropathic systemic amyloidosis.

Detailed Description Paragraph Right (143):
Gelsolin--Amyloids containing variants of gelsolin are associated with familial amyloidosis of Finnish type. Synthetic gelsolin peptides that have sequence homology to wildtype or mutant gelsolins and that form amyloid fibrils in vitro are reported in Maury, C. P. et al. (1994) Lab. Invest. 70:558-564. A nine residue segment surrounding residue 187 (which is mutated in familial gelsolin amyloidosis) was defined as an amyloidogenic region (Maury, et al., supra; see also Maury, C. P., et al. (1992) Biochem. Biophys. Res. Commun. 183:227-231; Maury, C. P. (1991) J. Clin. Invest. 87:1195-1199). Gelsolin or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of familial amyloidosis of Finnish type.

Detailed Description Paragraph Right (144):
Procalcitonin or calcitonin--Amyloids containing procalcitonin, calcitonin or calcitonin-like immunoreactivity have been detected in amyloid fibrils associated with medullary carcinoma of the thyroid (see e.g., Butler, M. and Khan, S. (1986) Arch. Pathol. Lab. Med. 110:647-649; Sletten, K., et al. (1976) J. Exp. Med. 143:993-998). Calcitonin has been shown to form a nonbranching fibrillar structure in vitro (Kedar, I., et al. (1976) Isr. J. Med. Sci. 12:1137-1140). Procalcitonin, calcitonin or a fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of amyloidosis associated with medullary carcinoma of the thyroid.

Detailed Description Paragraph Right (145):
Fibrinogen--Amyloids containing a variant form of fibrinogen alpha-chain have been found in hereditary renal amyloidosis. An arginine to leucine mutation at position 554 has been reported in amyloid fibril protein isolated from postmortem kidney of an affected individual (Benson, M. D., et al. (1993) Nature Genetics 3:252-255). Fibrinogen alpha-chain or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of fibrinogen-associated hereditary renal amyloidosis.

Detailed Description Paragraph Right (146):
Lysozyme--Amyloids containing a variant form of lysozyme have been found in hereditary systemic amyloidosis. In one family the disease was associated with a threonine to isoleucine mutation at position 56, whereas in another family the disease was associated with a histidine to aspartic acid mutation at position 67 (Pepys, M. B., et al. (1993) Nature 362:553-557). Lysozyme or a peptide fragment thereof that forms amyloid fibrils can be modified as described herein to create a modulator of amyloidosis that can be used in the detection or treatment of lysozyme-associated hereditary systemic amyloidosis.

Detailed Description Paragraph Right (159):
When natural .beta.-AP.sub.1-40 aggregation was assayed by this method in the absence of any .beta. amyloid modulators, high molecular weight aggregates were readily detectable on the gel. In contrast, when N-biotinyl-.beta.-AP.sub.1-40 modulator self-aggregation was assayed (i.e., aggregation of the N-biotinyl peptide alone, in the absence of any natural .beta.-AP), few if any high molecular weight aggregates were observed, indicating that the ability of the modulator to self-aggregate is significantly reduced compared to natural .beta.-AP. Finally, when aggregation of a mixture of natural .beta.-AP.sub.1-40 and N-biotinylated .beta.-AP.sub.1-40 was assayed by this method, reduced amounts of the peptide mixture associated into high molecular weight aggregates, thus demonstrating that the .beta. amyloid modulator is effective at inhibiting the aggregation of the natural D amyloid peptides.

Detailed Description Paragraph Right (160):
The neurotoxicity of the .beta.-amyloid modulators is tested in a cell-based assay using the neuronal precursor cell line PC-12, or primary neuronal cells, and the viability indicator 3,(4,4-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT). (See Shearman, M. S. et al. (1994) Proc. Natl. Acad. Sci. USA 91:1470-1474; Hansen, M. B. et al. (1989) J. Immun. Methods 119:203-210). PC-12 is a rat adrenal pheochromocytoma cell line and is available from the American Type Culture Collection, Rockville, Md. (ATCC CRL 1721). MTT (commercially available from Sigma Chemical Co.) is a chromogenic substrate that is converted from yellow to blue in viable cells, which can be detected spectrophotometrically.

Detailed Description Paragraph Right (197):
As shown in Table IV, cholyl-A.beta..sub.16-20 (PPI-350) and cholyl-A.beta..sub.17-21 (PPI-368) both exhibited inhibitory activity, indicating that the four-amino acid minimal subregion of positions 17-20 is sufficient for inhibitory activity. Loss of position 20 (e.g., in PPI-366 and PPI-321) resulted in loss of inhibitory activity, demonstrating the importance of position 20. Moreover, mutation of valine at position 18 to threonine (in PPI-369) also resulted in loss of activity, demonstrating the importance of position 18. In contrast, mutation of phenylalanine at position 19 to alanine (cholyl-A.beta..sub.16-20 Phe.sub.19- >Ala; PPI-370) resulted in a compound which still retained detectable inhibitory activity. Accordingly, the phenylalanine at position 19 is more amenable to substitution, preferably with another hydrophobic amino acid residue. Cholyl-penta-alanine; SEQ ID NO:35 (PPI-365) showed no inhibitory activity, demonstrating the specificity of the A.beta. peptide portion of the modulator. Moreover, unmodified A.beta..sub.16-20 (PPI-377) was not inhibitory, demonstrating the functional importance of the amino-terminal modifying group. The specific functional group influenced the activity of the modulator. For example, iminobiotinyl-A.beta..sub.16-20 (PPI-374) exhibited inhibitory activity similar to cholyl-A.beta..sub.16-20, whereas an N-acetyl neuraminic acid (NANA)-modified A.beta..sub.16-20 was not an effective inhibitory modulator (not listed in Table IV). A C-terminal amide derivative of cholyl-A.beta..sub.16-20 (PPI-319) retained high activity in delaying the lag time of aggregation, indicating that the carboxy-terminus of the modulator can be derivatized without loss of inhibitory activity. Although this